Instructions for Use

Laser Induced Fluorescence (LIF) Detector Manual

LIF Instructions for Use

PN 718113AF
January 2014

Beckman Coulter, Inc.
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BECKMAN COULTER

Laser Induced Fluorescence (LIF) Detector Manual
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PN 718113AF (January 2014)

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Revision History

This document applies to the latest software listed and higher versions. When a subsequent software version changes the information in this document, a new issue will be released.

Revision AF, 01/2014

Safety Notice

Read all product manuals and consult with Beckman Coulter-trained personnel before attempting to operate instrument. Do not attempt to perform any procedure before carefully reading all instructions. Always follow product labeling and manufacturer's recommendations. If in doubt as to how to proceed in any situation, contact your Beckman Coulter Representative.

Alerts for Danger, Warning, Caution, Important, and Note

⚠ WARNING

WARNING calls attention to a condition or possible situation that could cause injury to the operator.

⚠ CAUTION

CAUTION calls attention to a condition or possible situation that could damage or destroy the product or the operator's work.

IMPORTANT IMPORTANT is used for comments that add value to the step or procedure being performed. Following the advice in the Important adds benefit to the performance of a piece of equipment or to a process.

NOTE NOTE is used to call attention to notable information that should be followed during installation, use, or servicing of this equipment.

High Voltage Danger

This symbol indicates the potential of an electrical shock hazard existing from a high voltage source, and that all safety instructions should be read and understood before proceeding with the installation, maintenance, and servicing of all modules.

Do not remove system covers. To avoid electrical shock, use supplied power cords only and connect to properly grounded (three-hole) wall outlets. Do not use multi-plug power strips.

Laser Light

This symbol indicates that a potential hazard to personal safety exists from a laser source. When this symbol is displayed in this manual, pay special attention to the specific safety information associated with the symbol.

Laser Specifications

Laser Type	Class IIIa Laser Diode
Maximum Output	3 mW
Wavelength	635 nm

Laser Type	Class IIIb Argon-Ion Laser (air-cooled)
Maximum Output	3 mW
Wavelength	488 nm

Cautions and Warning Found in this Manual

Please read and observe all warnings and instructions. Remember, the user is the most important key to safety; operate this equipment with care.

CAUTIONS and WARNINGS found within this document are listed below.

⚠ WARNING

The instrument user may be able to select a laser device which may NOT have a power interrupt relay. The non-presence of a power-interrupt relay may defeat some of the Instrument safety interlock devices.

⚠ WARNING

Automated equipment can move without warning during operation. Keep hands and body away from moving components on the instrument.

⚠ CAUTION

Do not attempt to load or remove labware manually while the instrument is in operation.

⚠ WARNING

Do not spill liquids on or around the instrument. Wipe up any spills immediately.

⚠ WARNING

To avoid serious damage to the instrument, make sure that the laboratory site voltage/frequency matches the voltage/frequency that was ordered for the instrument.

⚠ WARNING

Do not attempt to remove or replace covers while the unit is powered on; disconnect power before removing or replacing the cover.

⚠ WARNING

Avoid direct exposure to the laser beam. Never look directly into the laser beam, and never leave the laser on, open, and unattended.

FCC Class A Compliance

This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to Part 15 of FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.

EMI Compliance

THIS DIGITAL APPARATUS DOES NOT EXCEED THE CLASS A LIMITS FOR THE RADIO NOISE EMISSIONS FROM DIGITAL APPARATUS AS SET OUT IN THE RADIO INTERFERENCE REGULATIONS OF THE CANADIAN DEPARTMENT OF COMMUNICATIONS.

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Laser Induced Fluorescence Detection

Specifications

Table 1 LIF Detection Specifications

Specification	Value
Relative Fluorescence Units (RFU) range	0 to 1000 RFU
Dynamic Range (at a dynamic range setting of 1000)	$> 10^4$
Sensitivity	1×10^{-11}M Sodium Fluorescein with a signal-to-noise ratio ≥ 2
Baseline Noise	< 0.005 RFU peak to peak[a]
Baseline Drift	< 0.2 RFU per hour[a]
488 nm Laser Module Dimensions	
Height	25.00 in. (63.5 cm)
Width	10.25 in. (26.3 cm)
Depth	14.25 in. (36.0 cm)
635 nm Laser Module Dimensions (Discontinued)	
Height	7.5 in. (19.2 cm)
Width	5.25 in. (13.2 cm)
Depth	7.0 in. (17.7 cm)
Wavelength Ranges (for optics)	
Excitation	300 to 700 nm
Emission	350 to 750 nm
Filters (optional)	520 nm bandpass filter
	488 nm notch filter
	675 nm long pass filter
Fiber cable length	6 feet (1.83 m)
Software compatibility	Instrument software, Version 2.0 or later
Laser (optional)	3 mW 488 nm (air-cooled) Argon Ion Laser
	635 nm Diode Laser

Table 1 LIF Detection Specifications *(Continued)*

Specification	Value
Power requirements	100/120V, 12A, 50/60 Hz
	or 220/240V, 6A, 50/60 Hz
Ambient Temperature Operating Range	15 to 40°C (39 to 104°F)
Recommended Best Performance Range	15 to 30°C (59 to 86°F)
Relative Humidity	<95% non-condensing @ 35°C (121°F)

a. These specifications are for a 75 µm I.D. capillary.

System Overview — LIF System

The LIF system consists of an instrument, along with the LIF Detector module, the LIF Interconnect Module, and a Laser Module. Refer to Figure 1 for more information. A regular instrument capillary cartridge with a LIF Detector plug installed is required for use with this system. The system requires the instrument software, version 2.0 or later.

The LIF Detector

The Laser Induced Fluorescence (LIF) Detector is available as an optional accessory. The LIF detector uses a laser light source. A 488 nm argon-ion laser is available from Beckman Coulter. Other lasers can be adapted. Refer to the section titled Using Other Lasers with the LIF Detector for more information. A fiber cable transmits excitation light from the laser to the capillary in the cartridge. Substances in the capillary which fluoresce at the laser wavelength are detected. The LIF Detector measures and records this fluorescence, which appears as a peak in the software.

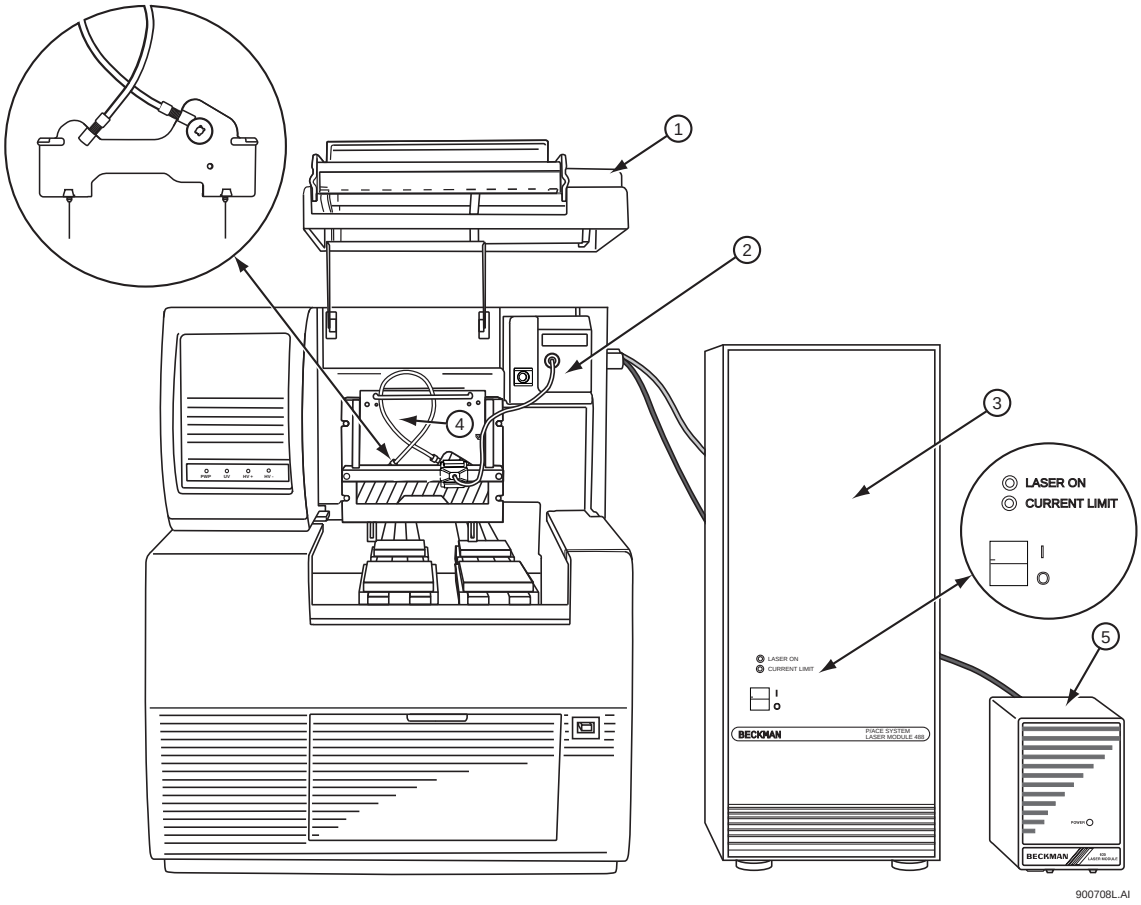
The initial installation of the LIF Detector must be performed by a Beckman Coulter Field Service Engineer. The instrument can easily be converted between LIF and UV/PDA modes as detector components are modular.

The LIF Detector can be used in the indirect mode. In this mode, a fluorescent buffer system is used to detect non-fluorescing components.

Component Description

The laser is used as the excitation source. The Interconnect Module is used to connect the laser to the instrument. A fiber optic cable and probe directs the laser light to the capillary. The LIF cartridge plug focuses the emitted light into the LIF Detector Module. The LIF detector collects the fluorescence emissions and converts it into an output signal. Filters are installed in the detector filter housing to correspond with the lasers and chemistry being used with the system.

Figure 1 Instrument with LIF Detector



1. Cartridge Cover
2. LIF Interconnect Module
3. 488 nm Laser Module
4. LIF Detector Module
5. 635 nm Laser Module (Discontinued)

The Laser Modules

The following section describes the optional 488 nm and 635 nm laser modules, and how they interface with the instrument and the LIF Detector. A complete set of laser operating instructions are also provided in this document.

⚠ WARNING

During normal operation of the LIF Detector, laser light is not accessible to the user. To prevent potentially harmful laser light from being emitted from the end of the fiber cable, an interlock mechanism turns off the laser if the laser fiber cable is disconnected from the interconnect module, or if the cartridge cover is opened.

Always power off the laser module and instrument before removing any of the LIF system module(s).

If these precautions should be defeated and the beam becomes accessible, DO NOT look into the beam or direct it toward someone else.

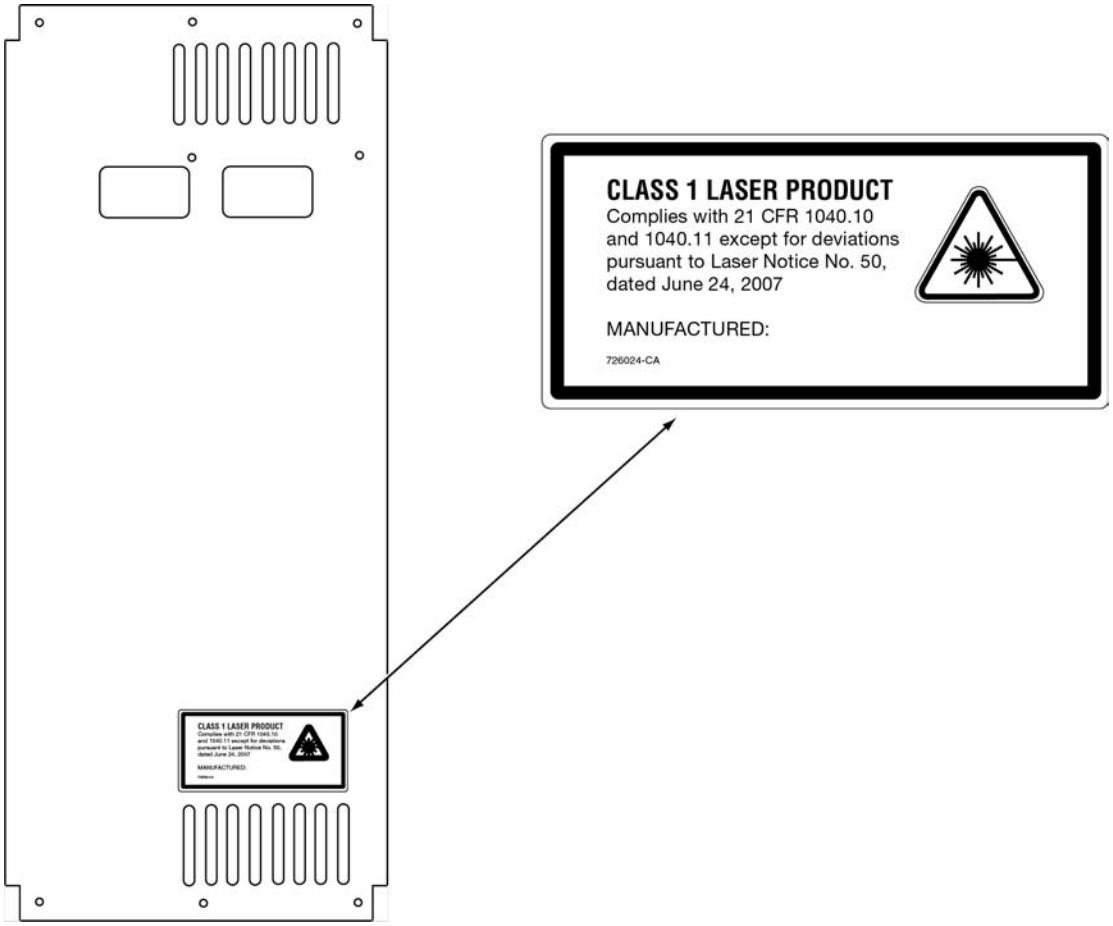
488 nm Argon Ion Laser Module

The Beckman Coulter 488 nm laser is an air-cooled Argon Ion Laser. Two indicator lights are located on the front of the 488 nm Laser Module. Refer to Figure 1 for more information. The green light flashes to indicate that the interlock circuit is complete and the laser is preparing to light (in approximately 40 seconds). Once the laser is operating, the green light will stay on continuously until the laser is turned off, the cartridge cover opened, or until the fiber cable coupler is disconnected from the LIF Interconnect Module.

The yellow light is a current limit indicator. A sensor in the laser module measures the amount of laser light being produced. Circuits in the laser module adjust the amount of current being drawn to maintain a stable 3mW output. When the laser tube is new, a fairly low current easily produces 3mW; as the laser tube ages, increasingly more current is needed to maintain the 3mW output.

The yellow indicator light will come on when the system begins to draw more than 7A of current. This light indicates that the laser tube should be replaced; when it comes on, contact Beckman Coulter Customer Service for assistance. Laser tube life should be longer than 2,000 hours.

Figure 2 488 nm Laser Module Back Panel



635 nm Laser Module

IMPORTANT This laser is discontinued and is no longer supported by Beckman Coulter.

The power switch is located on the back panel of the 635 nm laser housing. However, the laser will not light up unless the instrument is on, and all doors and interlocks are in place. A red light on the front panel of the 635 nm laser module indicates that the laser is on.

Using Other Lasers with the LIF Detector

Laser units other than the Beckman Coulter Laser Modules 488 and 635 can be used with the LIF detector, as long as the guidelines below are strictly followed.

- If you purchased your LIF detector without a Beckman Coulter Laser Module, an Interlock/Fiber Optic cable (P/N 360671) will have been provided. The custom interlock connector plugs into the LIF Interconnect Module. The fiber optic termination of the cable is the standard SMA 905. Your laser unit must be fitted with a fiber launcher which is compatible with this connector.

The two electrical wires in the cable connect to the interlock switches inside the detector and instrument. These switches close when it is safe for the laser to come on. These wires must be

connected to your laser's interlock circuit. The connectors and switches in the detector are rated for low voltage (TTL level) signals.

⚠ CAUTION

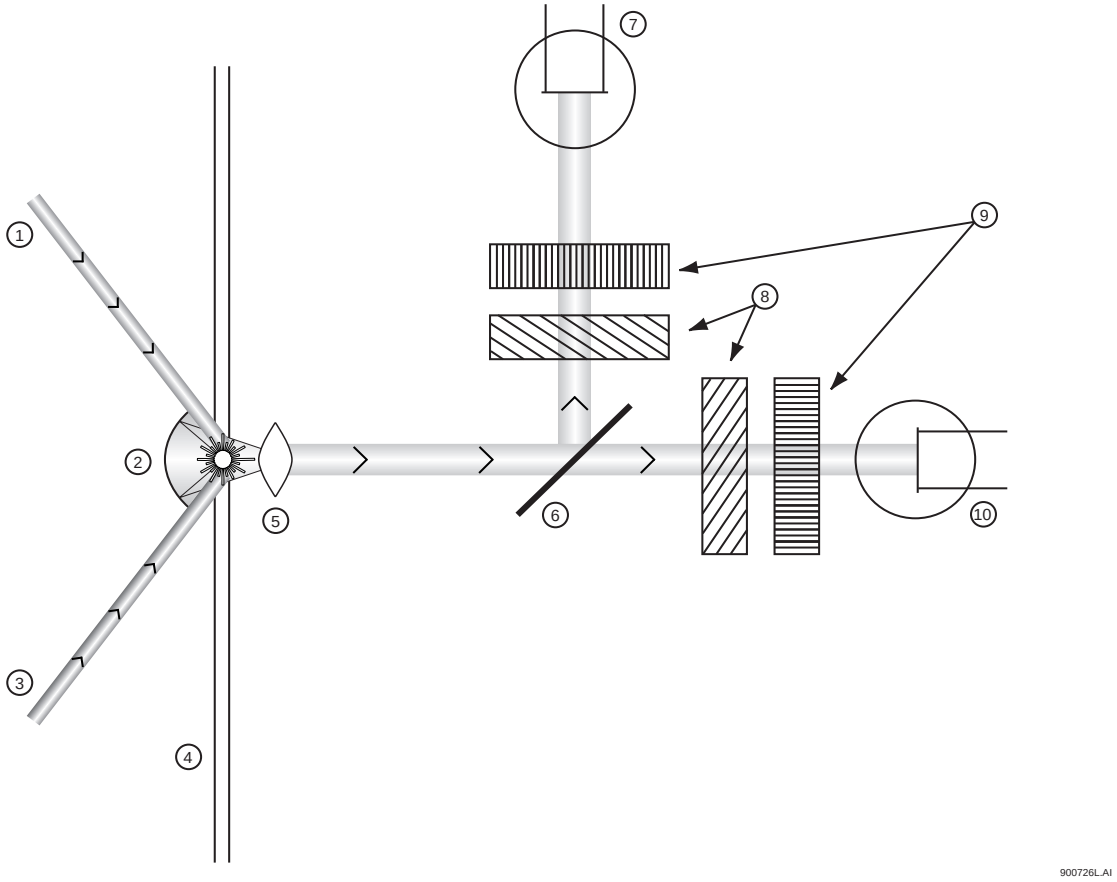
To avoid damaging the interlock connector and switches in the LIF Detector Module, do not connect interlock wires in the laser unit to a voltage greater than the rated voltage of the interlock connector and switches (5V, 0.1 A).

U.S. Federal regulations require that this connection be made to limit access to laser energy when the cable is disconnected from the LIF Interconnect Module.

Optical System

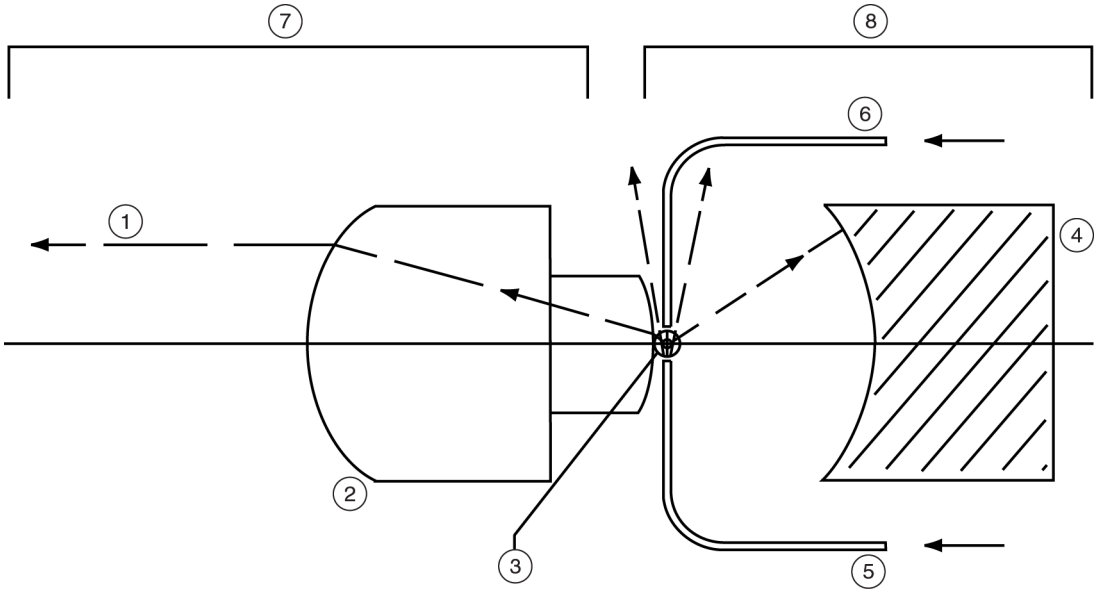
Refer to Figure 3 for a schematic overview of the LIF Detector optical diagram. Fiber optic cables carry laser light from the laser(s) through the interconnect module to the capillary window. When a fluorescent component passing through the capillary reaches the illuminated area, it emits light in all directions. This fluorescence is collected by a spherical reflector in the probe and a modified ball lens in the LIF Detector plug. The collected fluorescent light is split by a beam splitter and imaged onto two photomultiplier tubes, one for each channel. (For a single wavelength LIF detector, no beam splitter is present and the collected fluorescent light is imaged directly onto one photomultiplier tube).

Figure 3 LIF Optical System (Rotated 90° counterclockwise)



1. Laser 1
2. Spherical Mirror
3. Laser 2
4. Capillary
5. Ball Lens
6. Beam Splitter (50/50)
7. Photomultiplier Tube #2 (DETECTOR #2)
8. Laser Filters (Notch Filters)
9. Emission Filters
10. Photomultiplier Tube #1 (DETECTOR #1)

Figure 4 Optical Diagram



1. Collimated Collected Beam To Detector
2. Modified 5mm Dia. Sapphire lens (R_1 and R_2 = 2.5 mm)
3. Cross Section of the Capillary
4. Spherical Reflector
5. Excitation Fiber for Laser #1
6. Excitation Fiber for Laser #2
7. Plug
8. Probe

Installation

⚠ WARNING

Be sure to power down the instrument before proceeding with component installation.

Preinstallation Requirements

Initial installation, which will be done by a Beckman Coulter Field Service Engineer, involves setting up the laser, connecting it to the LIF Detector System, and verifying proper system operation (including operation of the laser interlock system, which prevents the user from exposure to laser light). The LIF Detector requires software and instrument firmware version 2.0 or later.

Bench Space

The instrument and the laser require an area of approximately four feet by two feet. Allow adequate space between instruments for air circulation (6 inch minimum). Additional space is required for a computer, printer, and other optional equipment, if applicable. Leave adequate space around the laser for air circulation.

⚠ CAUTION

Some laser modules produce significant quantities of heated air. Position these lasers so that this heated air is not drawn into the instrument.

Installing the Interconnect Module

⚠ WARNING

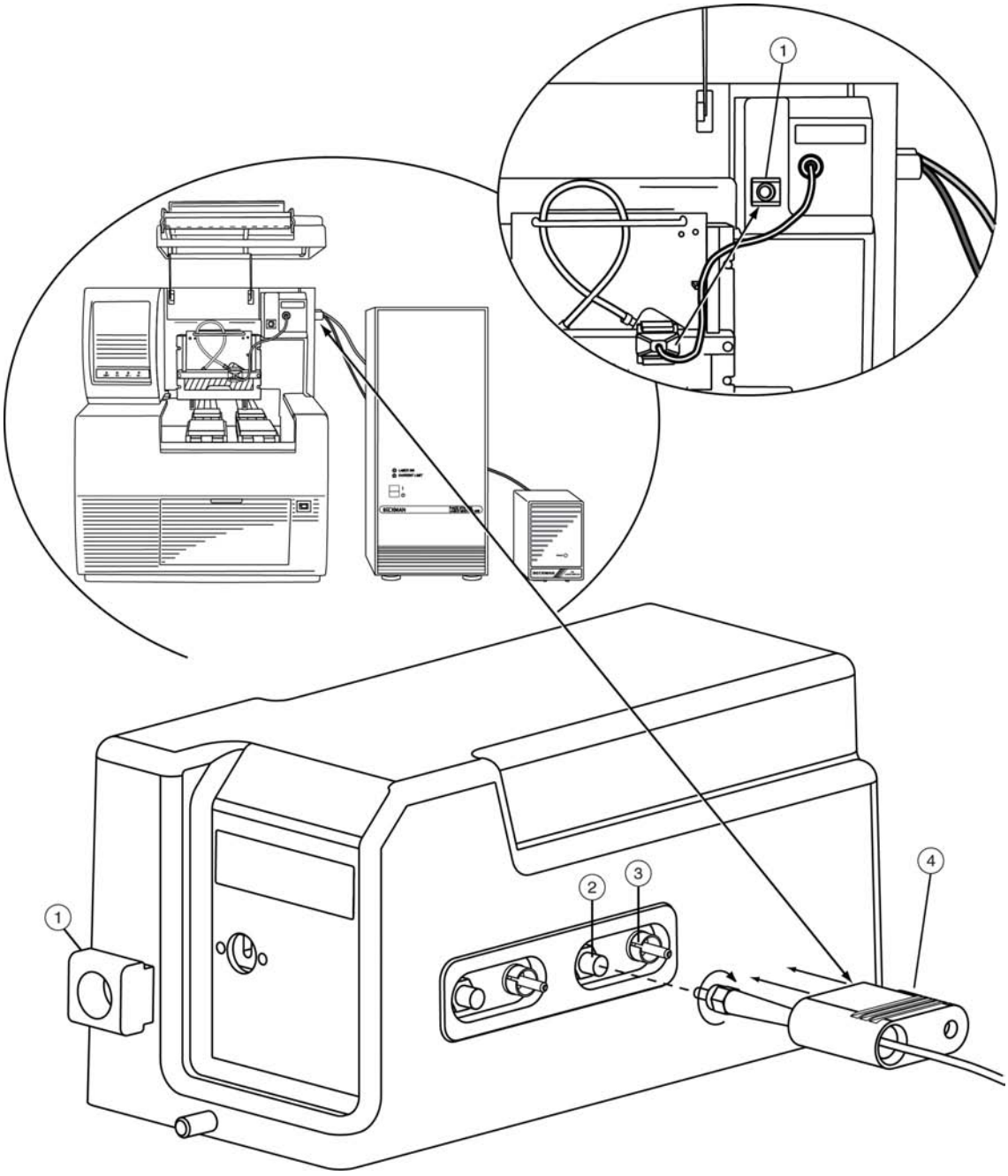
Be sure to power down the instrument before proceeding with component removal or installation.

If previously installed, remove the UV or PDA detector from the upper-right corner of the instrument. Carefully install the Interconnect Module in the upper-right corner. Secure the module by tightening the locking screw, located in the front lower middle area of this module, finger-tight.

Connecting the Laser Cable(s) to the Interconnect Module

Pull back the interlock cover, as shown in Figure 5. Hold the cable securely, and screw the fastener into the Interconnect Module. It is important not to allow either cable to become twisted during installation, as this may damage the fiber optic line inside the cable. Finally, install the interlock cover as shown in Figure 5.

Figure 5 Installing the Interconnect Module



1. Probe Holder
2. Fiber Optic Connector
3. Interlock Connector
4. Interlock Cover

Installing the LIF Detector Module

Prior to installing the LIF Detector, install the filter(s) and filter housing.

Installing Filters in the Filter Housing

The filter housing, which is inserted in the right side of the LIF Detector, has two filter holders, one for each detector channel. Refer to Figure 6 for a detailed diagram of the Filter Housing. One or more filters can be used in each filter holder, depending on the application and the width of the filter. During typical operation two filters are used: one laser filter and one emission filter. The laser filter prevents scattered laser light from passing into the detector. The emission filter is a band pass filter that allows only the fluorescence signal at a selected wavelength to pass into the detector.

Beckman Coulter supplies the following filters:

- 520 nm, 560 nm and 675 nm emission
- 488 nm laser filter (notch filter)

Depending on the application and laser, you may use any standard 0.5 inch diameter optical filter in the range of 350 to 750 nm.

To install filter(s) into the filter holder (refer to Figure 6):

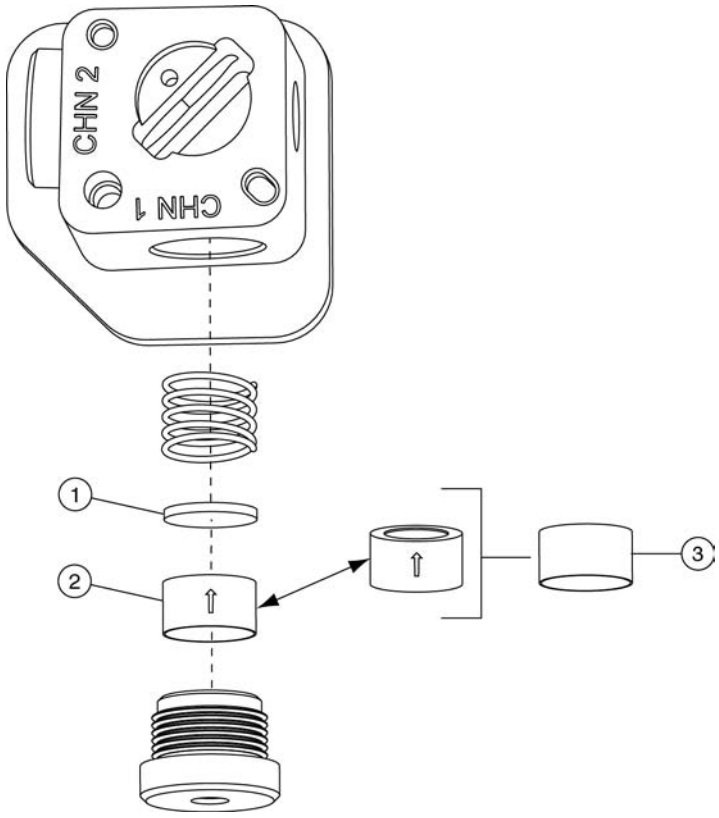
1. Hold the filter housing in the same orientation as shown in the drawing.
2. Remove the beam blocker if present.
3. Hold the filter with the arrow on the outside edge of the filter pointing up.
   (If the filter does not have an arrow on its edge, refer to the note below). *Be sure to handle filters by the edges.*
4. Carefully place the filter into the filter holder.
5. When all filters are in the filter holder, insert the spring and install the filter holder in the appropriate location (channel 1 or 2) in the filter housing.

6 If the filter(s) are removed from a channel, re-install the beam blocker in its place to protect the photomultiplier tube.

NOTE If there is no arrow on the edge of the filter you are using, look at both sides of the filter and determine which side has the more reflective surface. (Typically the more reflective side also has a wide edge rim that extends beyond the filter surface). The filter should be installed with the more reflective side facing the light source (the laser). In the LIF detector, laser light comes from the center of the filter housing. So if you are holding the housing in the proper orientation, install a filter with the arrow and/or reflective side with the wider rim pointing up (toward the filter housing).

If a filter is installed incorrectly, it will work but its performance may not be optimal.

Figure 6 Installing Filters in the Filter Housing

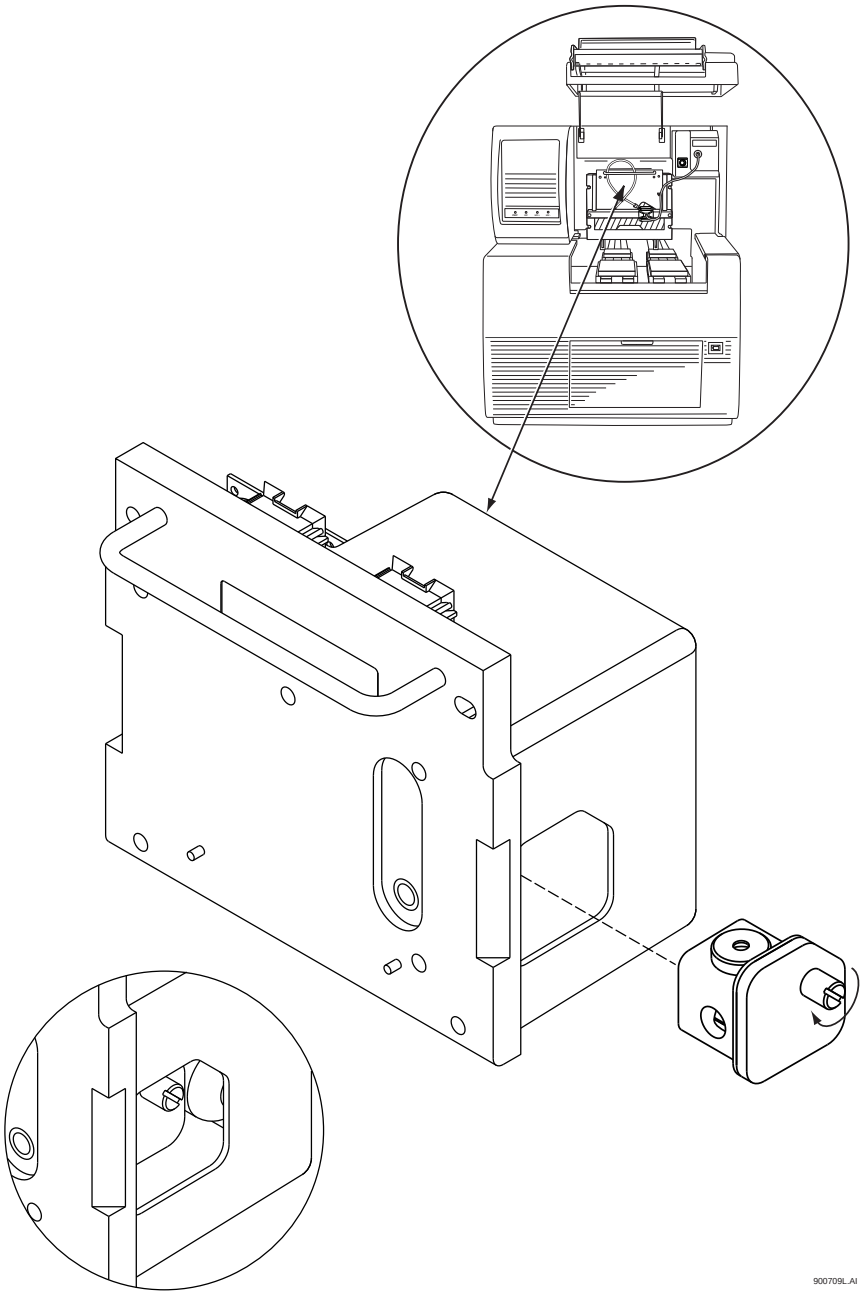


1. Laser Filter
2. Emission Filter
3. Beam Blocker — use when filters are not installed

Installing the Filter Housing into the Detector

Insert the filter housing into the opening in the right side of the detector. Refer to Figure 7 for more information. Make sure the “keyed” corner of the filter housing lines up with the “keyed” corner of the Detector Module. The housing will fit into the opening in this orientation only, so it cannot be installed incorrectly.

Figure 7 Installing the Filter housing into the LIF Detector



Installing the LIF Detector into the Instrument

⚠ WARNING

Be sure to power down the instrument before proceeding with component installation.

Hold the LIF Detector level and place it into the instrument, as shown in Figure 7, keeping the two female connectors on the LIF Detector level to the two male connectors located on the instrument. Secure the Detector Module in place by fastening the captive screws to finger tightness.

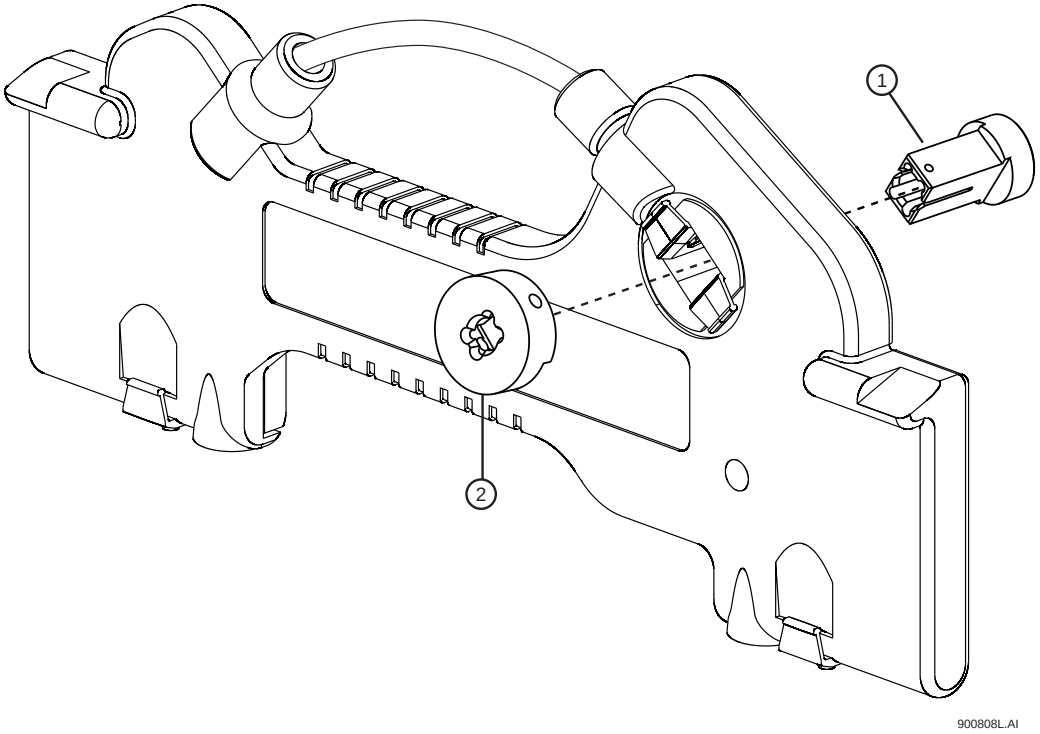
Preparing the Cartridge for LIF Usage

Build the cartridge as described in the Installation and Maintenance Manual, up to the point when the aperture plug is installed. The LIF Detector uses a special plug, which is not interchangeable with those used on the UV/PDA system.

To install the LIF plug and probe stabilizer (see Figure 8):

1. Carefully install the metal LIF plug from the back of the cartridge.
2. Snap on the probe stabilizer such that the lock pins snap into the dimples on the plug.

Figure 8 Installing the LIF Plug and Probe Stabilizer



1. LIF Plug
2. Probe Stabilizer

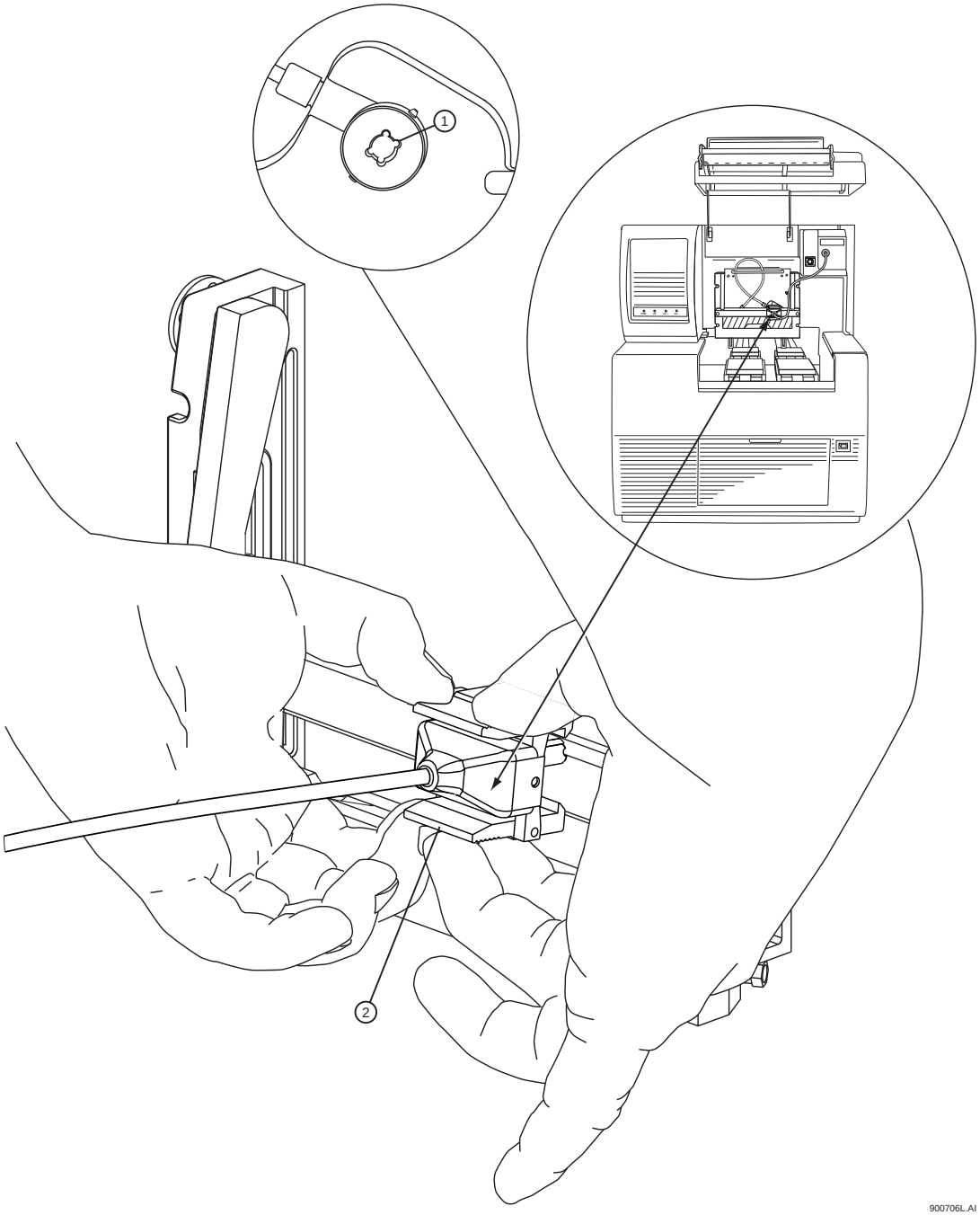
Install the cartridge containing an LIF plug into the instrument. Refer to section 3 of the Installation and Maintenance Manual for more information on this procedure.

Connecting the Probe to the Cartridge

When connecting the probe to the LIF Cartridge, align the two pins on the left side of the probe to the corresponding openings on the clamping bar. Squeeze the probe clamp to open the jaws on the probe, and carefully install the probe onto the clamping bar. Refer to Figure 9 for more information on this procedure.

NOTE When not in use, install the probe in the Probe Holder (see Figure 5) on the Interconnect Module. This provides a mechanical protection for the probe and shields it from dust and other particles.

Figure 9 Connecting the Probe to the LIF Cartridge



1. Probe Stabilizer
2. Probe Clamp

Operation

Preparation

⚠ WARNING

Make sure the laser fiber optic cable and interlock devices are connected to the LIF Detector before turning on power to the instrument or the laser. If the interlock is defeated, laser light could be accessible in the interior or emitted from the fiber optic cable.

1 Follow the instructions described in the Installation section before proceeding with step 2.

2 Turn on the instrument.

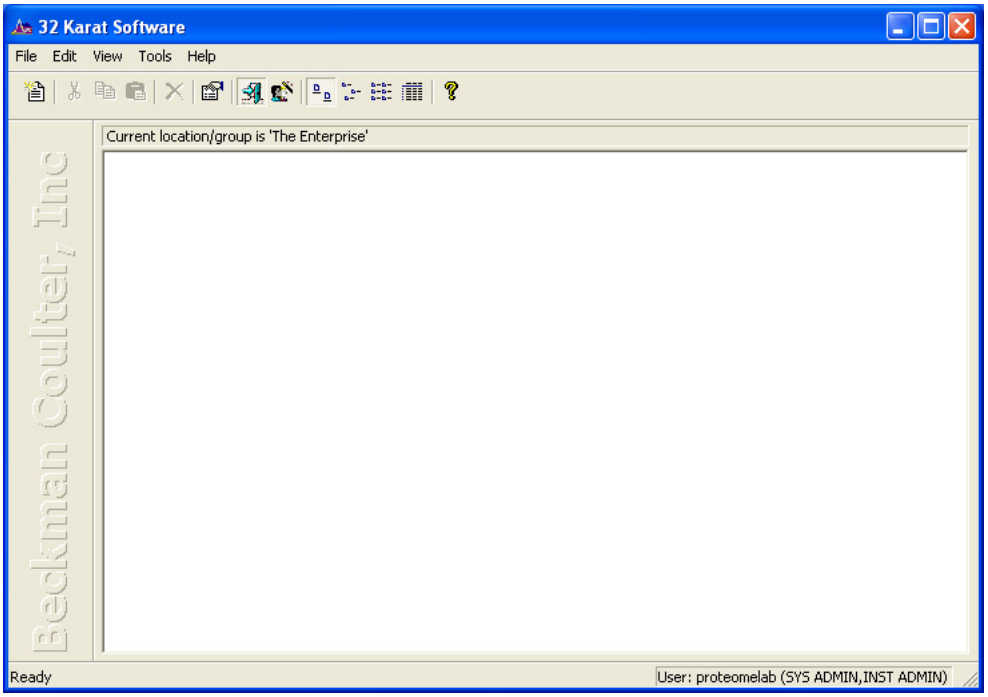
3 Turn on the laser. After the laser lights, wait at least 15 minutes for the laser output to stabilize.

NOTE For instructions on operating the instrument from 32 Karat software, see the *P/ACE MDQ User's Guide*.

Configuring 32 Karat for LIF Operation

1 The 32 Karat Enterprise screen is shown in Figure 10. Right-click in this screen to create a new instrument. Right-click, then click **New > Instrument**.

Figure 10 Main Menu



2 Name the new instrument. Right-click the newly created instrument to configure, then click **Configure > Instrument . . .** See Figure 11 and Figure 12.

Figure 11 Configure Instrument menu command

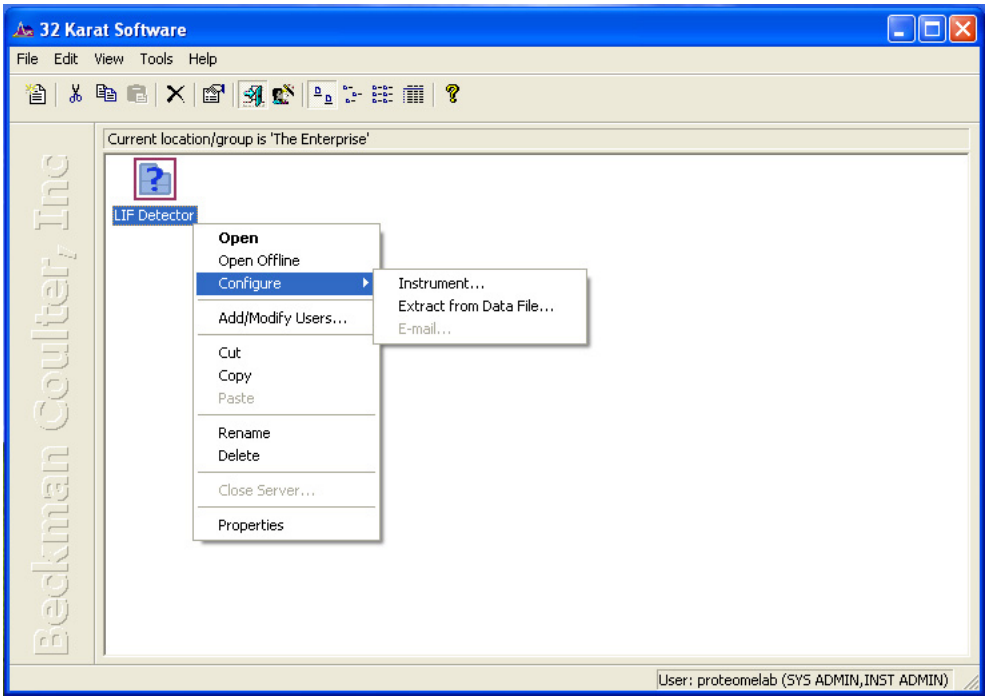
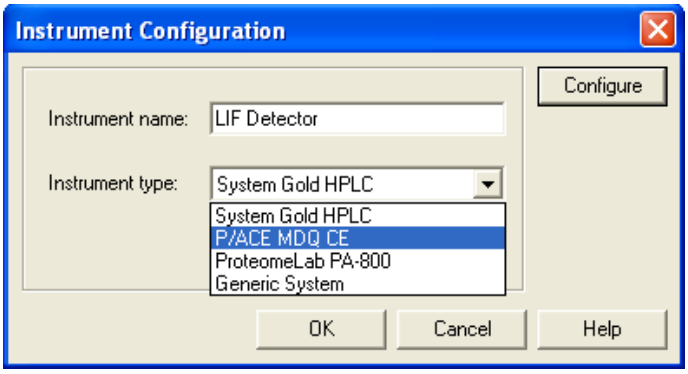


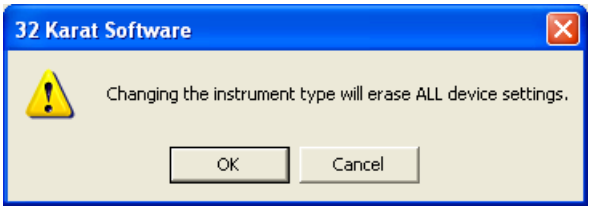
Figure 12 Choosing the Type of Instrument



3 Choose the type of instrument you want to control, P/ACE MDQ CE or ProteomeLab PA 800.

A warning notice appears stating that changing the instrument type will erase ALL device settings. Since this is a new instrument configuration and there is no data to lose, choose **OK** to continue. Then click **Configure**.

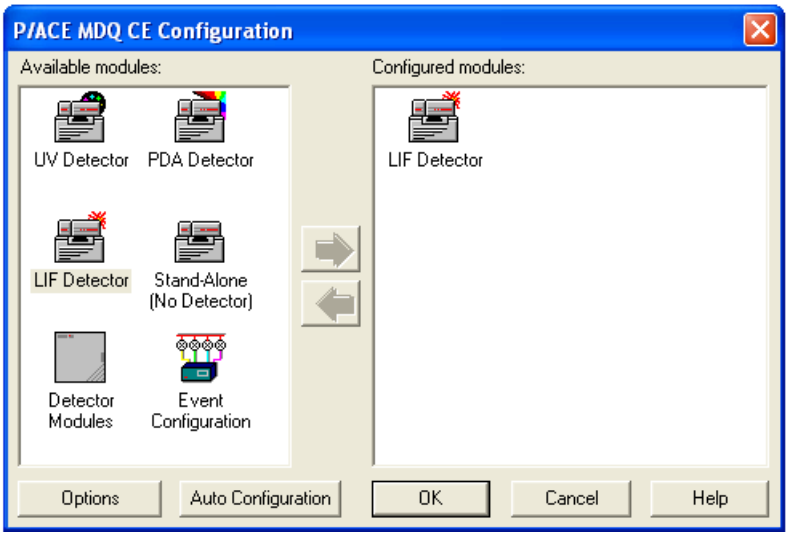
Figure 13 Confirmation



There are two options for configuring an instrument, manual or auto configure. Manual configuration enables the user to customize the instrument configuration. Auto Configuration will detect the type of detector installed and then scan the trays to determine what is installed. The instrument needs to be powered on with the desired trays installed for Auto Configure to work.

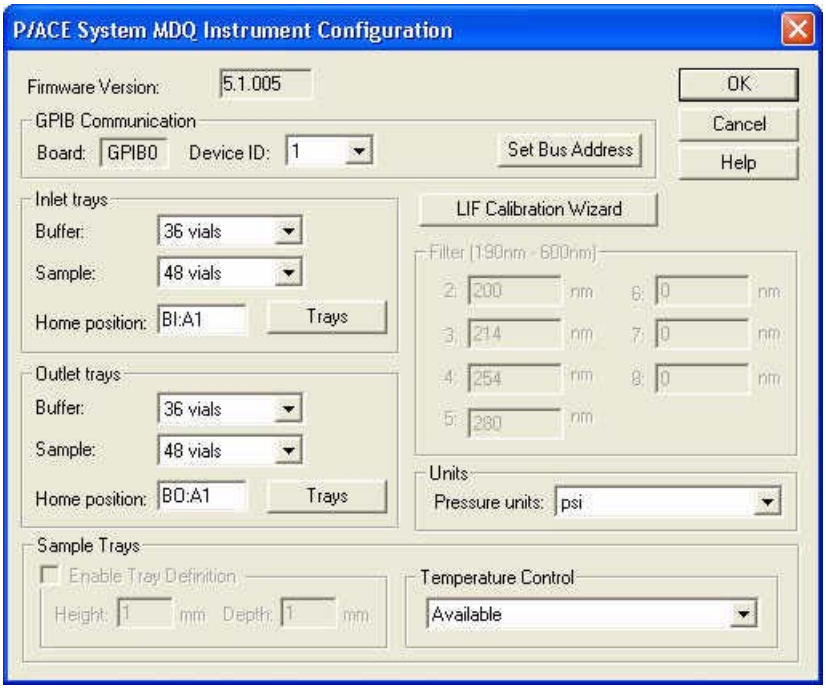
4 Double-click on the LIF Detector on the right side of P/ACE MDQ CE Configuration Screen. See Figure 14.

Figure 14 Choosing the LIF Detector



The P/ACE System MDQ Instrument Configuration screen allows the user to define the types of trays installed, define the home position of each tray, identify the firmware version and access the LIF Calibration Wizard.

Figure 15 Configuring the LIF Detector



5 Choose **OK** to accept all the parameters set up in the configuration.

Direct Control

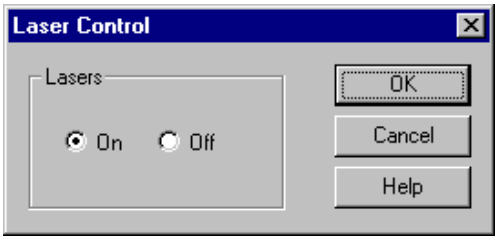
Figure 16 shows the Direct Control screen for instruments configured with an LIF Detector.

Figure 16 Direct Control Screen

1 Clicking the laser symbol on the Direct Control screen brings up the Laser Control dialog box, shown in Figure 17. Select **ON** to indicate that the lasers are on.

NOTE The lasers cannot be controlled individually.

Figure 17 Laser Control Screen



Laser Control

The lasers can be controlled from Direct Control or by adding the 'Laser ON' or 'Laser OFF' event to the time program section of a method. Both lasers are switched simultaneously and cannot be controlled individually.

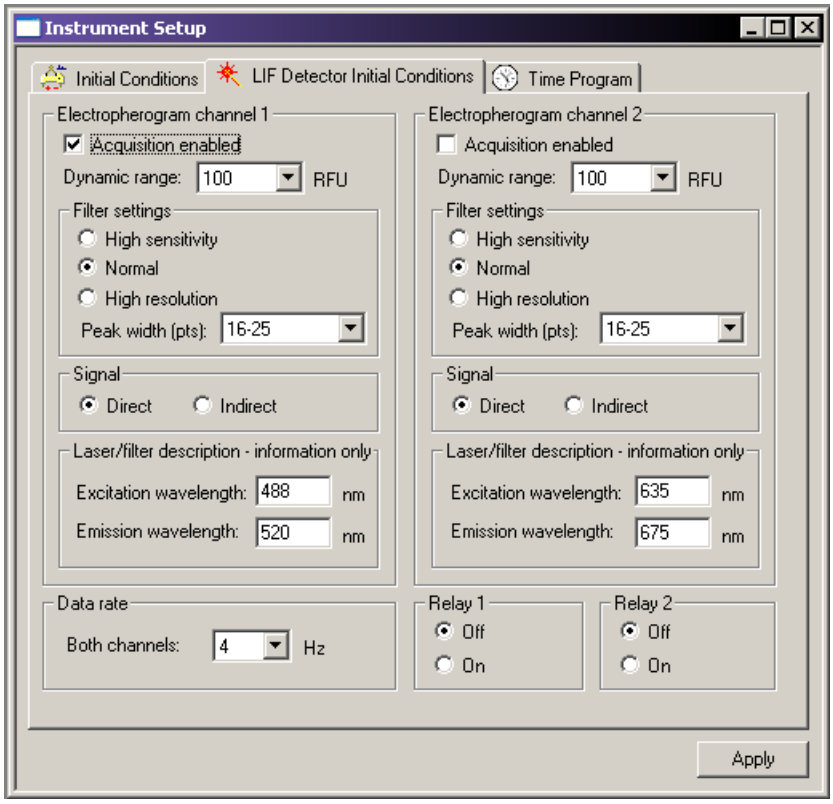
The laser control is a software-controlled on/off switch which enables or disables the laser interlock. The laser has to be physically powered on for this control to be functional. The

status on the Direct Control and Status screen reflects the position of this on/off switch and **not** the state of the laser.

Time Program

When programming a method with LIF Detection, select **Method > Instrument Setup**. The instrument setup window will appear. See Figure 18.

Figure 18 LIF Detector Initial Conditions Screen



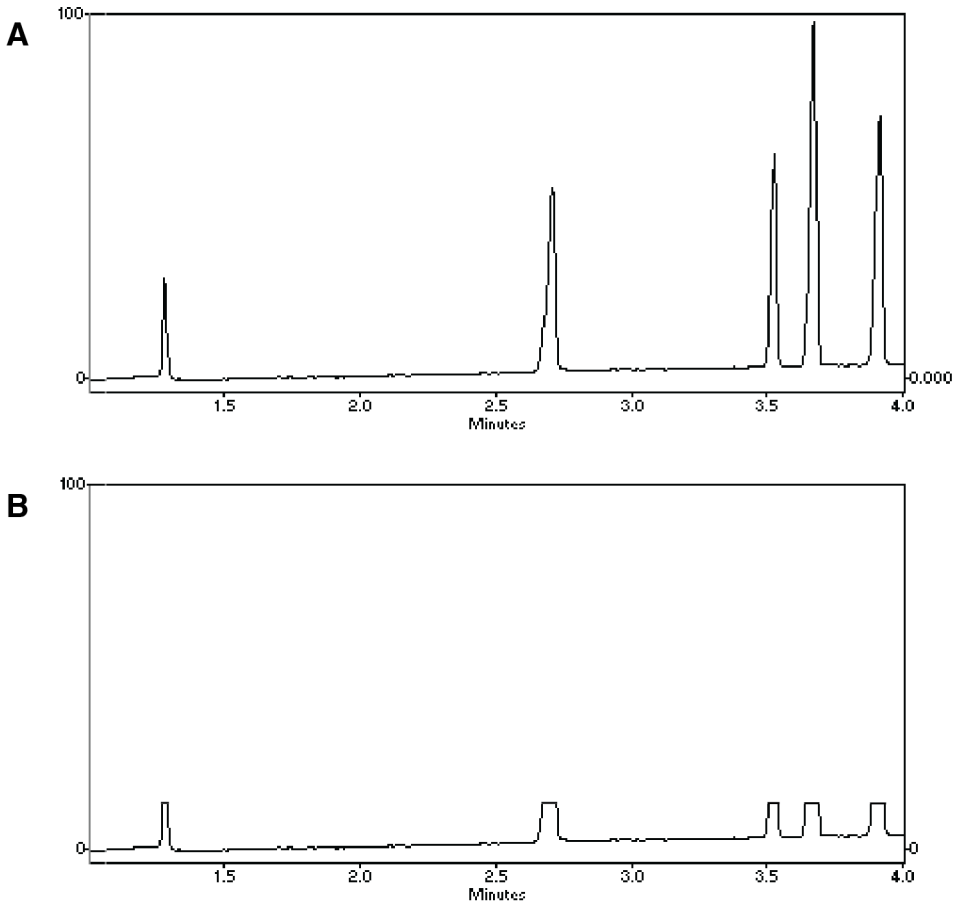
NOTE Excitation and Emission Wavelength settings are for indication purposes only. The excitation and emission wavelengths are determined by the laser and filter combination installed in the LIF detector.

Dynamic range: A Dynamic range of 1.0 through 1000 RFU can be selected.

Dynamic Range

In the *IEEE Standard Dictionary of Electrical and Electronics Terms*, the dynamic range is defined as "The range of output levels that can be read, from saturation level to the level of minimum discernible output signal." For the LIF detector, the upper limit of the dynamic range corresponds to the maximum output signal from the detector (the highest RFU value detectable). If a dynamic range is selected which is lower than the largest peak, peak tops will be truncated and flattened (see Figure 19).

Figure 19 Electropherograms (A, B) with different Dynamic Ranges



A. Electropherogram with Dynamic Range set to 100.

B. Same Electropherogram with Dynamic Range set to 10. Note that the heights of all peaks have exceeded the dynamic range setting and were truncated.

For optimal results, select a dynamic range slightly larger than the tallest peak in the electropherogram.

NOTE The higher the dynamic range, the higher the relative noise level in the electropherogram. This effect is not significant at dynamic range settings of ≤ 100, but is clearly visible at a dynamic range setting of 1000. For optimal sensitivity, use a dynamic range setting of ≤ 100, even if this would involve dilution of the sample prior to injection.

Calibration Correction Factor

Unlike absorbance detectors, there is no absolute reference point for fluorescence detectors; measurements are reported relative to a defined "standard" in relative fluorescence units (RFU). This measurement is dependent on a number of parameters including but not limited to:

- Capillary internal diameter
- Concentration of the fluorescent species

- Fluorescence yield of the molecule being measured
- Background buffer composition and properties
- Separation parameters (temperature, voltage, etc.)
- Laser power

To minimize variation caused by (small) changes to any of the parameters listed above, a correction factor can be used to normalize the detector response against a defined standard.

The Calibration Correction Factor (CCF) is the mathematical relationship between the fluorescence from a known concentration of the analyte and the associated signal detected by the instrument. Subsequent measurements are multiplied by the factor to determine the relative fluorescence unit.

For the LIF Detector system, a Calibration Wizard is available to simplify the determination of the calibration correction factor. During the CCF determination, the Calibration Wizard instructs the instrument to execute the following:

- Measure the background (with buffer)
- Measure the signal from the “standard”

The Calibration Wizard will then:

- Calculate the change in signal between the standard and the background buffer
- Calculate the correction factor using the following equation:

$$CCF = \frac{\text{Target RFU Value}}{\text{Measured RFU Value}} \bullet CCF_O$$

where:

Target RFU value = Target value for the standard solution used (entered by user into the Wizard).

Measured RFU value = Change in signal between background buffer and standard.

CCF_O = The current value of the Calibration Correction Factor (CCF).

Once the CCF value is calculated and accepted by the user, all subsequent measurements will be multiplied by this value.

As long as run conditions, capillary I.D. and chemistries remain unchanged, the CCF calculated should remain valid. It is recommended that the standard solution be measured (as a check) periodically or when a new capillary is installed.

NOTE The Calibration Correction Factor determined in this process will affect all subsequent analysis.

⚠ CAUTION

The LIF System must be installed and lasers must be warmed up according to the laser manufacturer's instructions before running the Calibration Wizard.

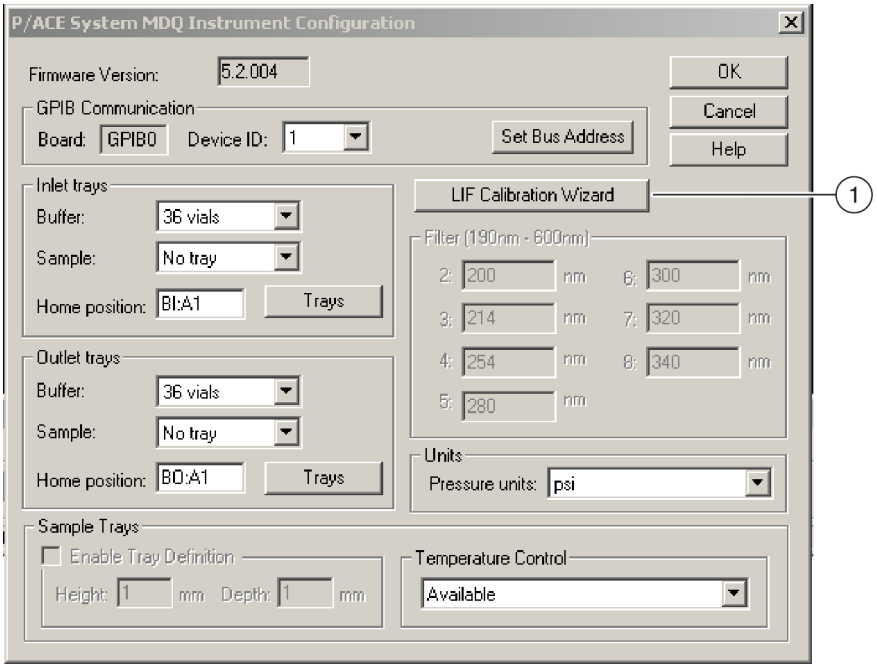
Calibration Wizard

To access the Calibration Wizard

1. Right-click on the LIF instrument from the 32 Karat home screen.
2. Choose **Configure > Instrument** and click the **Configure** button.
3. Double-click on the LIF Detector icon in the Configured modules window.

4 Click on the **LIF Calibration Wizard**. See Figure 20.

Figure 20 Accessing the LIF Calibration Wizard



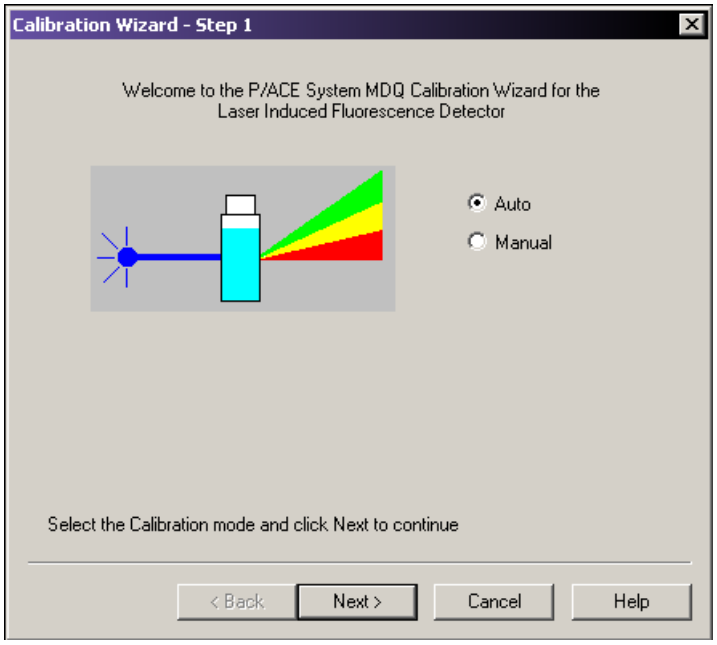
1. LIF Calibration Wizard button

Calibration Wizard – Step 1

After starting the Calibration Wizard, the **Calibration Wizard – Step 1** screen is displayed as shown in Figure 21. The following options are presented:

- Auto
- Manual

Figure 21 Calibration Wizard – Step 1


Calibration Wizard - Step 1
Welcome to the P/ACE System MDQ Calibration Wizard for the Laser Induced Fluorescence Detector
Auto
Manual
Select the Calibration mode and click Next to continue
< Back
Next >
Cancel
Help

Selecting Auto Mode will guide the user through a series of steps that will automatically determine the appropriate value for the calibration correction factor (CCF).

Select Auto Mode, and click the **Next** button. The **Calibration Wizard - Step 2** screen appears, as shown in Figure 22.

NOTE Manual Calibration is discussed in the section Manual Mode.

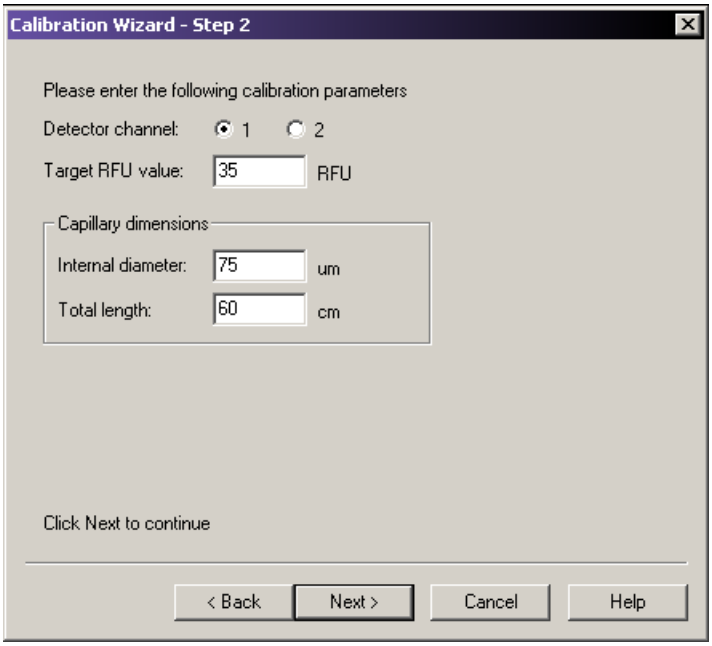
Calibration Wizard – Step 2

1 Enter the following calibration parameters: (See Figure 22.)

- Detector Channel 1 or 2 (note that the Calibration Wizard must be run **separately** for each channel)
- Target RFU Value (the value expected for the standard)
- Capillary Dimensions – Internal Diameter (in µm), and Total Length (in cm)

2 Click **Next**.

Figure 22 Calibration Wizard – Step 2

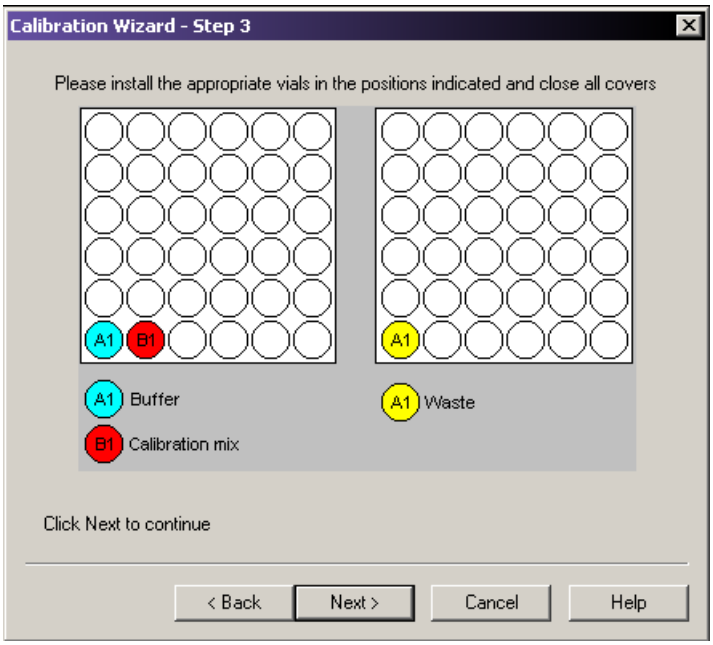


Calibration Wizard – Step 3

The following Calibration Wizard – Step 3 screen appears, as shown in Figure 23.

1 Load the buffer and calibration mix as shown in Figure 23.

Figure 23 Calibration Wizard – Step 3



NOTE The Buffer and Calibration mixture must be appropriate for the analysis to be run. It is recommended to use 2.0 ml buffer vials with red caps for this procedure.

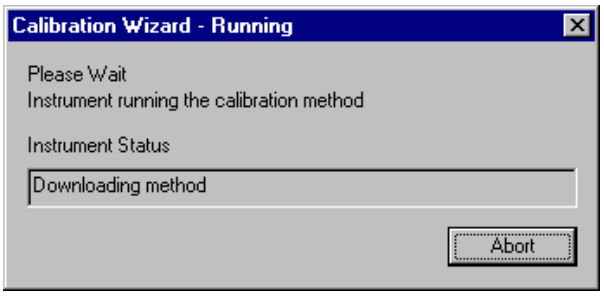
2 After installing the vials in the tray, close the instrument lid and click **Next**.

The screen shown in Figure 24 is displayed while the calibration is running. Please wait until the instrument has completed its calibration, which may take several minutes.

⚠ WARNING

DO NOT open the instrument doors during this step.

Figure 24 Calibration Wizard Running Screen



Calibration Wizard – Step 4

When the Calibration is completed, the calculated value (or CCF) for the channel selected is displayed. Clicking **Accept** will cause this value to be used during subsequent analysis. If **Cancel** is selected, the previously stored value will be retained.

Figure 25 Calibration Wizard – Step 4 Screen

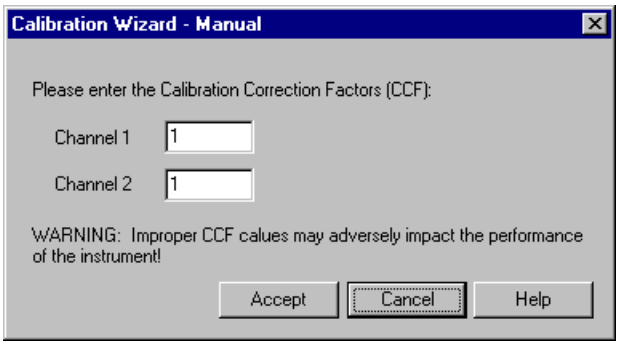
Manual Mode

The Manual Mode allows the user to enter values for the Calibration Correction Factor manually.

When Manual Mode is selected in Step 1 (see Figure 21), the screen shown in Figure 26 is displayed. Enter the desired CCF values, and click **Accept**. These values will be used for all subsequent analysis.

NOTE Running the Calibration Wizard in the Auto Mode is preferred for optimum performance.

Figure 26 Calibration Wizard – Manual Mode



Care and Maintenance

Introduction

⚠ WARNING

Any maintenance procedure requiring removal of an instrument panel exposes the operator to the possibility of electrical shock and/or mechanical injury. Such service procedures should only be done by trained, qualified personnel.

The Beckman Coulter 488 nm and 635 nm laser modules operate at high voltages and emits laser light that can cause serious damage to the eyes. Servicing of the laser should be done only by a Beckman Coulter Field Service Representative.

Inspecting the System

The LIF detector system is designed to prevent laser light exposure outside the laser box, fiber cable, and detector. To ensure continued laser light containment, regularly check the following parts of the system.

- Visually inspect the full length of the fiber cable to make sure it is in good condition.
- Visually inspect the laser module housing to verify that no panels are loose, which could provide access to laser energy inside the module.
- Verify operation of the interlock. **If the interlock is defeated, potentially harmful laser power of up to 3mW could be accessible in the interior or could be emitted from the fiber.**

Cleaning

Clean the exterior surfaces of the detector and laser module regularly by wiping with a clean, damp cloth. If necessary, a mild detergent can be used; finish by wiping with a soft, dry cloth.

The filters are delicate optical components that must be protected from dirt, dust, and fingerprint contamination. Once installed in the filter housing, they are protected from contamination and normally do not require cleaning. Do NOT clean a filter unless it is dirty enough to affect system performance, due to the risk of damaging the filter during the cleaning process.

Storage

When not in use, install the probe in the Probe Holder on the Interconnect Module. This provides a mechanical protection for the probe and shields it from dust and other particles.

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